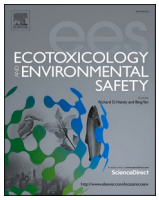
Ecotoxicology and Environmental Safety 246 (2022) 114129

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Petroleum pollution affects soil chemistry and reshapes the diversity and networks of microbial communities

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ARTICLE INFO

Edited by: R Pereira

Keywords:
Soil
Petroleum
Microorganism
Metabolism

ABSTRACT

Soil is the bearing centre of terrestrial ecosystems. Oil pollution leads to changes in the physical and chemical properties of soil to varying degrees. Polluted soils form a unique microbial species composition, which provides rich materials for the bioremediation of oil-contaminated soil through biological enhancement. Understanding the microbial composition of petroleum-contaminated soil can provide a better biological method for soil remediation. Based on this, 16 S rRNA and ITS genetic markers were used to analyse the bacterial and fungal microbiota in petroleum-contaminated soil, and their physical and chemical properties (total organic carbon, alkaline hydrolysable nitrogen, total phosphorus, total potassium, available potassium, Cu, Zn, and Cd) were measured. It was found that petroleum pollution can significantly reduce the abundance and diversity of bacteria and fungi in the soil and significantly promote the relative abundance of *Proteobacteria, Pseudomonas, Pseudoxanthomonas* and *Pseudoallescheria*, which changed the dominant flora of bacteria and fungi and reshaped the co-occurrence network relationship between bacteria and fungi in oil-contaminated soil. The content of total organic carbon in petroleum-contaminated soil was significantly higher than that in uncontaminated soil, while the content of alkaline hydrolysable nitrogen and available potassium was significantly lower than that in uncontaminated soil, and the content of Cu significantly increased after pollution. Total organic carbon is the key driving factor that changes oil-contaminated soil microorganisms and plays a significant role in regulating the remodelling and composition of the microbial community in oil-contaminated soil. This study laid a solid theoretical foundation for the bioremediation of oil-contaminated soil.

1. Introduction

Petroleum is a complex mixture of organic matter composed of a large number of hydrocarbons (n-alkanes, polycyclic aromatic hydrocarbons, etc.), a small number of nonhydrocarbon compounds (sulfides, nitrides, alkane acids, etc.) and organic metals (vanadium, nickel, etc.) (Li et al., 2021a; Wang et al., 2020). Due to the leakage in the process of oil exploitation, smelting, storage and transportation, as well leakage and bursts of oil well pipelines, the problem of oil pollution is on the rise (Varjani et al., 2020; Zuzolo et al., 2021). Soil is the bearing centre of the terrestrial ecosystem. Petroleum pollutants mainly exist on the surface of the soil (Li et al., 2020c), which will cause the physical properties = (porosity, moisture content, etc.)) and chemical properties (pH, total organic carbon (TOC), etc.) of the soil to change to varying degrees; for example, the loose spaces of the soil become compact, the water content and oxygen content are reduced, the nutrient circulation slows down, and the original structure of the soil is changed, thereby affecting the permeability of the soil, the dispersion and combination of soil particles, the composition of soil organic matter, and the combination of organic matter and soil particles (Ali et al., 2020; Galitskaya et al., 2021; Pacwa-Pociniczak et al., 2020). Petroleum pollutants contain many organic groups. When petroleum enters the soil environment, its rich compounds combine with inorganic nitrogen and phosphorus in the soil, thus limiting the nitrification and dephosphorization of the soil and resulting in the reduction of quick-acting nitrogen and phosphorus in the soil. The organic matter in petroleum also greatly increases the organic carbon content in the soil, changes the ratio of soil carbon to nitrogen, and affects soil fertility, which is the main reason the composition and content of organic matter in the soil surface changes. These changes in physical and chemical properties will reduce material exchange between plant roots and soil (Borowik et al., 2019; Li et al., 2020b).

In addition, there are abundant microbial communities in the soil.

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https://doi.org/10.1016/j.ecoenv.2022.114129
Received 22 November 2021; Received in revised form 22 September 2022; Accepted 26 September 2022
Available online 30 September 2022
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The richness and evenness of microbial communities determine the diversity of soil microbial species. The structure of microbial communities has a strong correlation with soil parent materials, environmental factors and crop planting conditions (Mafiana et al., 2021). Soil bacteria play an important role in the carbon cycle, inorganic salt cycle and plant growth (Hereira-Pacheco et al., 2021; Nam et al., 2021; Zhu et al., 2021). Fungi are the second largest microbial group in soil, and there are tens of thousands of fungi in 1 g soil (Newsham et al., 2020; Tan et al., 2021). Generally, microorganisms play a key role in the soil ecosystem, material cycle and energy flow in the biosphere (Singha and Pandey, 2021). Soil microorganisms are an important part of soil biological activity and participate in decomposition, transformation and other biochemical processes of organic matter in soil. Studies have shown that petroleum components have toxic effects on many soil microorganisms. Therefore, after petroleum pollutants infiltrate into the soil, the number of active soil microorganisms is significantly reduced, and the microbial community and microflora are changed, resulting in a decrease in the function of microorganisms in the soil environment, a decrease in soil activity, and even some loss (Cruz et al., 2019; Liu et al., 2020).

At present, oil-contaminated soil has seriously affected the economic development and ecological environment of global oil fields. The remediation of contaminated soil has become the focus of current environmental research and one of the major environmental problems. To solve this problem, we must fully understand the impact of oil pollution on soil. Therefore, it is of great significance to study the changes in microbial community structure and function in oil-contaminated soil (Li et al., 2021b).

In summary, this study focuses on the analysis of bacterial community diversity in oil-contaminated soil and its correlation with environmental factors. By measuring the physical and chemical properties of oil-contaminated soil and surrounding control soil and analysing the bacteria and fungi in each group, the unique bacterial composition and abundance of contaminated and unpolluted soil were obtained; these results provide abundant material for the bioremediation of petroleum-contaminated soil by bioaugmentation. This effective exploration of environmental factors and soil microbial community structure has laid a solid theoretical foundation for the bioremediation of oil-contaminated soil.

2. Materials and methods

2.1. Sample collection

The soil samples collected in the experiment were from Qingcheng County, and the loess high crude oil wellhead and nearby soil samples were collected in Huachi County and Xifeng District, located in an area 107.4° − 108° E, 35.7° − 36.8° N. As the main oil producing area of the Changqing Oilfield, the Loess Plateau in Eastern Gansu has caused 500–1000 hm^2 of oil pollution in the process of oil exploitation. The content of soil crude oil in the heavily polluted area is as high as 3510 mg/kg, seven times higher than the critical value (500 mg/kg).

A total of 30 petroleum-contaminated topsoil samples (0–20 cm) were collected as experimental soil, and 30 uncontaminated topsoil samples were collected nearby as control soil (soil samples with a total petroleum hydrocarbon content less than 500 mg/kg were regarded as clean soil). The sand and gravel on the soil surface were removed before sampling. The sampling point was marked (the distance between each sampling point is more than 100 m) as a square with a side length of 20 cm; the soil was excavated at the marked spot with a soil shovel (using the five-point sampling method); 0–20 cm of shallow soil was collected at each sampling point; approximately 30 g of soil sample was taken at each point; the soil obtained from each sampling point was fully mixed; and the soil samples were sieved using a 2 mm pore size filter. Each soil sample was divided into two parts, one for the determination of environmental factors and one for amplicon sequencing. Each collected soil sample was placed into a 50 mL sterile centrifuge tube, marked and stored in an icebox. After air drying, the collected soil samples were stored at − 80 ℃. All soil samples were air-dried and fully mixed after removing pebbles and plant residues. Prior permission was granted from the competent authority for sampling, and the field study did not involve any endangered or protected species.

2.2. Determination of soil physical and chemical properties

The total amounts of copper and zinc in the soil were determined by hydrochloric acid, nitric acid and perchloric acid digestion methods, and the quantitative filtrate to be measured was obtained by constant volume dilution. The contents of Cu and Zn in the solution to be tested, the blank digestion solution and the standard series solution were determined by atomic absorption spectrometry. Preparation of Cu and Zn standard curve: the same operating parameters as the sample determination were used, the absorption value (A) of Cu and Zn standard solutions were measured with the atomic absorption spectrometer, and the concentration was prepared. A standard curve of absorption value (A) or linear regression equation were used. The calculation formula:

$$\text{total Cu (or Zn) content in soil } (mg{\cdot}kg^{-1}) = (\rho - \rho 0) \times V/m$$

where ρ - the mass concentration of Cu or Zn in the solution to be measured by the standard curve ($\mu g{\cdot}mL^{-1}$);.

$\rho 0$ - the mass concentration of Cu or Zn in the blank digestive solution found by the standard curve ($\mu g{\cdot}mL^{-1}$);.

V - constant volume after digestion (mL);.

M - mass of dried soil sample (g).

Cd in the soil was extracted by the aqua regia perchloric acid digestion method, and the quantitative filtrate to be measured was obtained by constant volume dilution. The conditions of the atomic absorption spectrometer were set, and the solution was sprayed directly into the air acetylene flame to determine the absorption value.

Preparation of the Cd standard curve: Different volumes of Cd standard solution were placed into different volumetric flasks, 0.5 mL aqua regia and 1.5 mL perchloric acid were added, and the volume was fixed with HCl solution to prepare a series of Cd standard solutions with different concentrations. The standard curve of the concentration absorption values was drawn (A). Calculation formula:

$$\text{Total cadmium (Cd) content in soil } (mg{\cdot}kg^{-1}) = \rho \times V \times ts/(m \times k)$$

where ρ - mass concentration of cadmium in the solution (μg·mL-1).

V - volume.

ts - division multiple.

m - mass of air-dried sample (g);.

k - moisture coefficient.

TOC measurement was conducted by the Walkley–Black (WB) method (Walkley and Black, 1934). Alkali hydrogenated nitrogen (AHN) was extracted or digested with 0.5 M H_2SO_4-6 M HCl-1 M H_2SO_4 and H_2SO_4-$HClO_4$-0.5 M $NaHCO_3$-2.0 M KCl. Samples were then analysed using a continuous flow analytical system (SKALAR SAN^{++}, Netherlands) (China EPA Method 350, 2007). Total nitrogen (TN) was determined using the Kjeldahl method (Bremmer, J. M. (1996). Nitrogen Total. In D. L. Sparks (Ed.). Methods of soil analysis, Part 3: chemical methods (pp.1085–1121). Madison: Soil science society of America). The total phosphorus (TP) in the soil was determined by NaOH melting aluminium antimony anti colorimetry. Through the reaction with the aluminium antimony anti chromogenic agent, it was quantitatively determined by spectrophotometry. The content of total potassium in the soil was determined by the NaOH melting flame photometric method in the national standard method (GB-7854–87).

2.3. DNA extraction, PCR amplification, and sequencing

Microbial DNA extractions were performed with the MP Bio Fast DNA Spin Kit for soil (SDS/mechanical lysis). The 515 forward (5′-

GTGCCAGCMGCCGCGG-3′) and 806 reverse (5′-GGACTACHVGGGTWTCTAAT-3′) primers were used to amplify the V4 region of the 16 S rDNA gene, and the SSU0817 forward (5′-TTAGCATGGAATAATRRAATAGGA-3′) and 1196 reverse (5′-TCTGGACCTGGTGAGTTTCC-3′) primers were used to amplify the ITS1-F region of the 18 S rDNA gene. The library was sequenced on an Illumina NovaSeq 6000 platform.

2.4. Bioinformatics and statistical analyses

Paired-end reads were assigned to samples based on their unique barcodes and were truncated by cutting off the barcodes and primer sequence. Paired-end reads were merged using FLASH (version 1.2.11) to obtain the raw tags. Quality filtering on the raw tags were performed using the fastp (version 0.20.0) software to obtain high-quality clean tags. The clean tags were compared with the reference database (Silva database for 16 S, Unite database for ITS) using Vsearch (version 2.15.0) to detect the chimera sequences, and then the chimera sequences were removed to obtain the effective tags. For effective tags, OTU (operational taxonomic unit) clustering was performed with 97% identity using Uparse (v7.0.1001) software.

According to the results of species annotation, the top 10 species with the highest abundance of each group at each taxonomic level (phylum and genus) were selected to generate a column accumulation diagram of species relative abundance, and the species with higher relative abundance and their proportion were analysed. Alpha diversity was used to analyse the diversity of microbial communities in the samples. The observed species, Chao1, Shannon, Simpson, ACE, and Good coverage indices were calculated by using QIIME software (version 1.7.0) to evaluate the species richness and diversity differences of the microbial communities in each sample. Principal coordinate analysis (PCoA) analysis was performed on samples by using the "vegan" package and the "ggplot2" package in R software to reflect the differences among samples. The biomarkers with significant differences were found by random forest analysis using the "randomForest" package in R (Zhang et al., 2018). The "igraph" package was used to analyse the cooccurrence

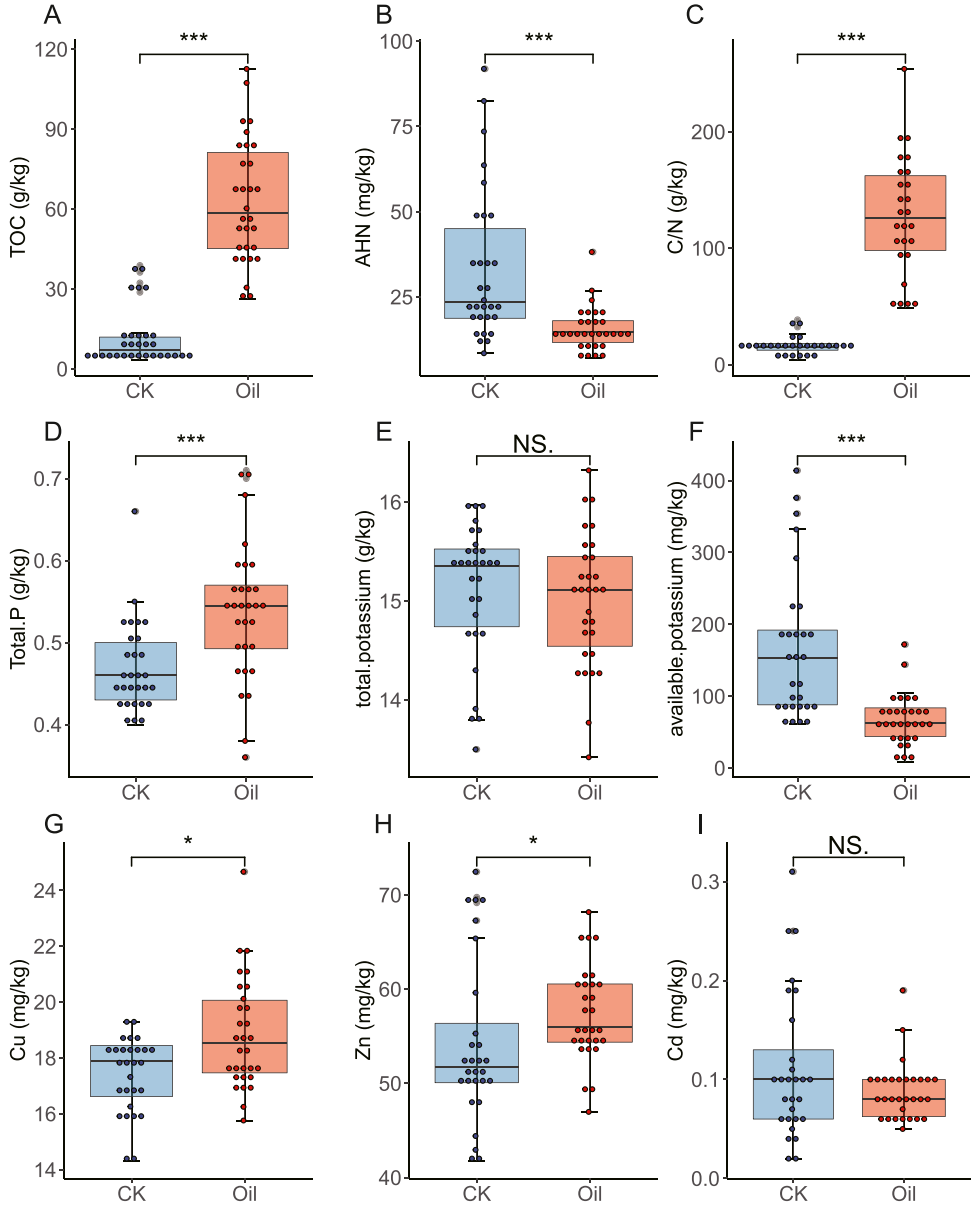

Fig. 1. Determination of physical and chemical indices in petroleum-contaminated soil. A-I represent the TOC (g/kg), AHN (mg/kg), C/N ratio, total P (g/kg), total potassium (g/kg), available potassium (mg/kg), Cu (mg/kg), Zn (mg/kg) and Cd (mg/kg) contents in the CK and Oil groups, respectively. The data were analysed by the Wilcoxon test (*p < 0.05; **p < 0.01; ***p < 0.001). AHN: *alkali hydrogenated nitrogen.* CK: *control group;* Oil: *Oil pollution soil group*, the following are the same.

network of the bacterial community structure, and Gephi 0.9.2 software was used to draw the network map (Csardi and Nepusz, 2006). Based on Spearman correlation analysis and Mantel test analysis, the correlation between environmental factors and flora was analysed, and the p value of significance was obtained.

3. Results

3.1. Changes in soil physic and chemistry by petroleum pollution

We measured the physical and chemical indices of the soil and found that the TOC content in group Oil was significantly higher than that in group CK (Fig. 1A; $P < 0.001$), the AHN (alkali hydrogenated nitrogen) content was significantly lower than that in group CK (Fig. 1B; $P < 0.001$), the C/N ratio in group Oil was significantly higher than that in group CK (Fig. 1C; $P < 0.001$), the total P content was significantly higher than that in group CK (Fig. 1D; $P < 0.001$), the total potassium content was not significantly different from that in group CK (Fig. 1E), and the available potassium content was significantly lower than that in group CK (Fig. 1F; $P < 0.001$). The contents of Cu and Zn in the Oil group were significantly higher than those in the CK group (Figs. 1G, 1H; $P < 0.05$), and the content of Cd in the Oil group was not significantly different from that in the CK group (Fig. 1I). The above results indicate that petroleum contamination will cause a significant increase in TOC, C/N ratio, total P, Cu and Zn content in the soil while reducing the content of AHN, alkaline hydrolysed nitrogen and available potassium.

3.2. Effect of petroleum pollution in the diversity of soil microorganisms

The diversity of the bacterial microorganisms in the CK group and the Oil group was explored through 16 S rRNA sequencing technology. We found that Good's coverage for the two groups was greater than 97.5%, indicating a great sequencing depth for the analysis of bacteria (Fig. 2 A). At the same time, the Good's coverage index in the Oil group was significantly higher than that in the CK group ($P < 0.001$). For alpha-diversity analysis, Chao 1 estimators and Shannon indices were used to assess community richness and diversity, respectively. The Chao 1 index of the CK group was significantly higher than that of the Oil group ($P < 0.001$), which indicates that the richness of bacteria in the CK group was higher than that of the oil group; that is, the richness of bacteria in the soil was significantly reduced due to oil pollution (Fig. 2B). In addition, the Shannon index of the CK group was significantly higher than that of the Oil group (Fig. 2C; $P < 0.001$), which indicated that the bacterial diversity of the CK group was significantly higher than that of the Oil group; that is, the diversity of bacterial microorganisms in the soil was significantly reduced after oil pollution. In conclusion, oil pollution significantly reduces the richness and diversity of bacterial microorganisms in soil.

We also sequenced and analysed the fungi in the oil-contaminated soil and found that the Good's coverage for the two groups was greater than 99.0%, indicating a great sequencing depth for the analysis of fungi (Fig. 2D). In addition, the Chao 1 index of the CK group was also significantly higher than that of the Oil group (Fig. 2E; $P < 0.001$), which indicated that the richness of fungi in the CK group was higher than that of the Oil group; that is, the abundance of fungi in the soil also decreased significantly after oil contamination. The Shannon index of the CK group was significantly higher than that of the Oil group, indicating that the fungal diversity of the CK group was significantly higher than that of the Oil group (Fig. 2 F; $P < 0.001$); that is, the fungal diversity in the soil was significantly reduced after oil pollution. In conclusion, these results indicate that petroleum pollution can significantly reduce the abundance and diversity of bacteria and fungi in soil.

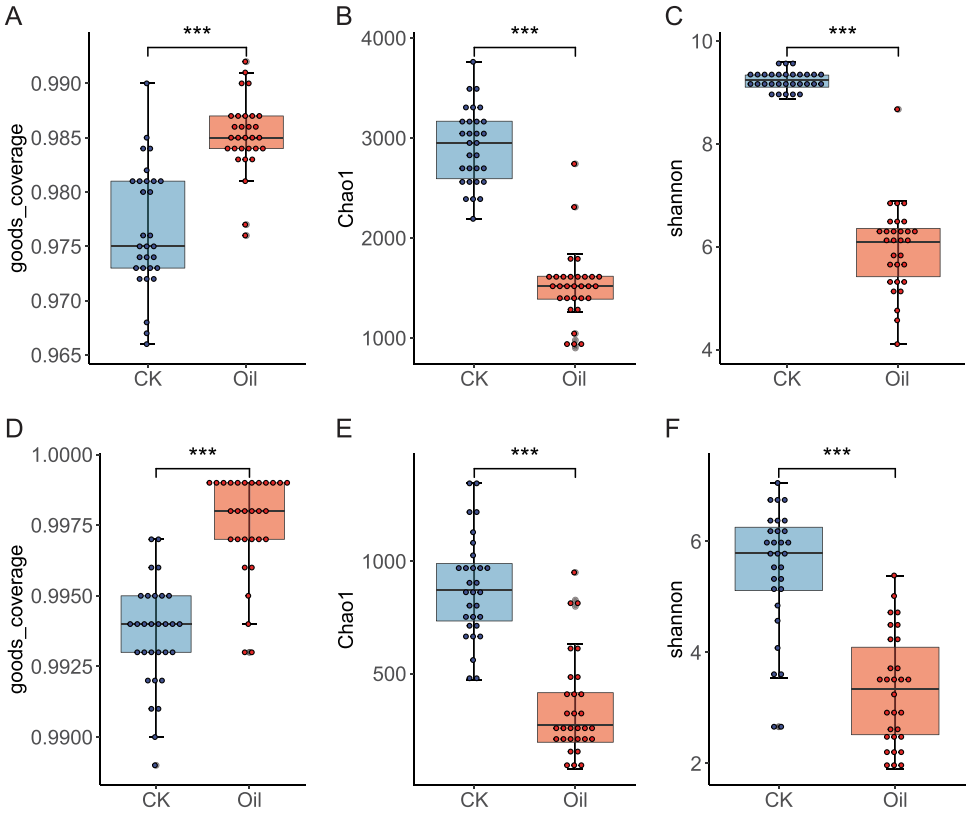

Fig. 2. α-Diversity of bacteria and fungi in petroleum-contaminated soil. C Good's coverage, Chao1 and Shannon index of bacteria for the Oil and CK groups. The significant differences between groups were calculated by T tests. D-F Good's coverage and Chao1 and Shannon indices of fungi for the Oil and CK groups. The significant differences between groups were calculated by the Wilcoxon test (***$p < 0.001$).

3.3. Composition and change in microbial community structure in oil-contaminated soil

To measure the extent of the similarity between bacterial microorganism communities in the two groups, principal coordinate analysis (PCoA) plots of weighted distance were generated (Fig. 3A). The significant differences between groups were calculated by analysis of similar (ANOSIM) tests. From the PCoA analysis, we found that there was a significant difference in bacterial microorganism distribution between the CK and Oil groups. Among these algorithms, the PC1 distribution with the largest contribution rate revealed changes of 52.18%, and the PC2 distribution revealed changes of 10.03%. The composition of bacterial microorganisms in group CK tended to be the same, while those in group Oil tended to be the same. The fact that the composition of samples in the group is consistent, the difference is small, and there is obvious regularity supports accuracy of the results and indicates that oil contaminated soil will significantly change the composition of overall bacteria in the soil. To explore the specific distribution and composition of bacterial flora in oil-contaminated soil, we further analysed the composition of the flora at the phylum level (Fig. 3B) and the genus level (Fig. S1). The bacterial microbiota of the CK group was composed of 34.58% *Proteobacteria*, 12.7% *Actinobacteria*, 17.2% *Bacteria*, 9.3%

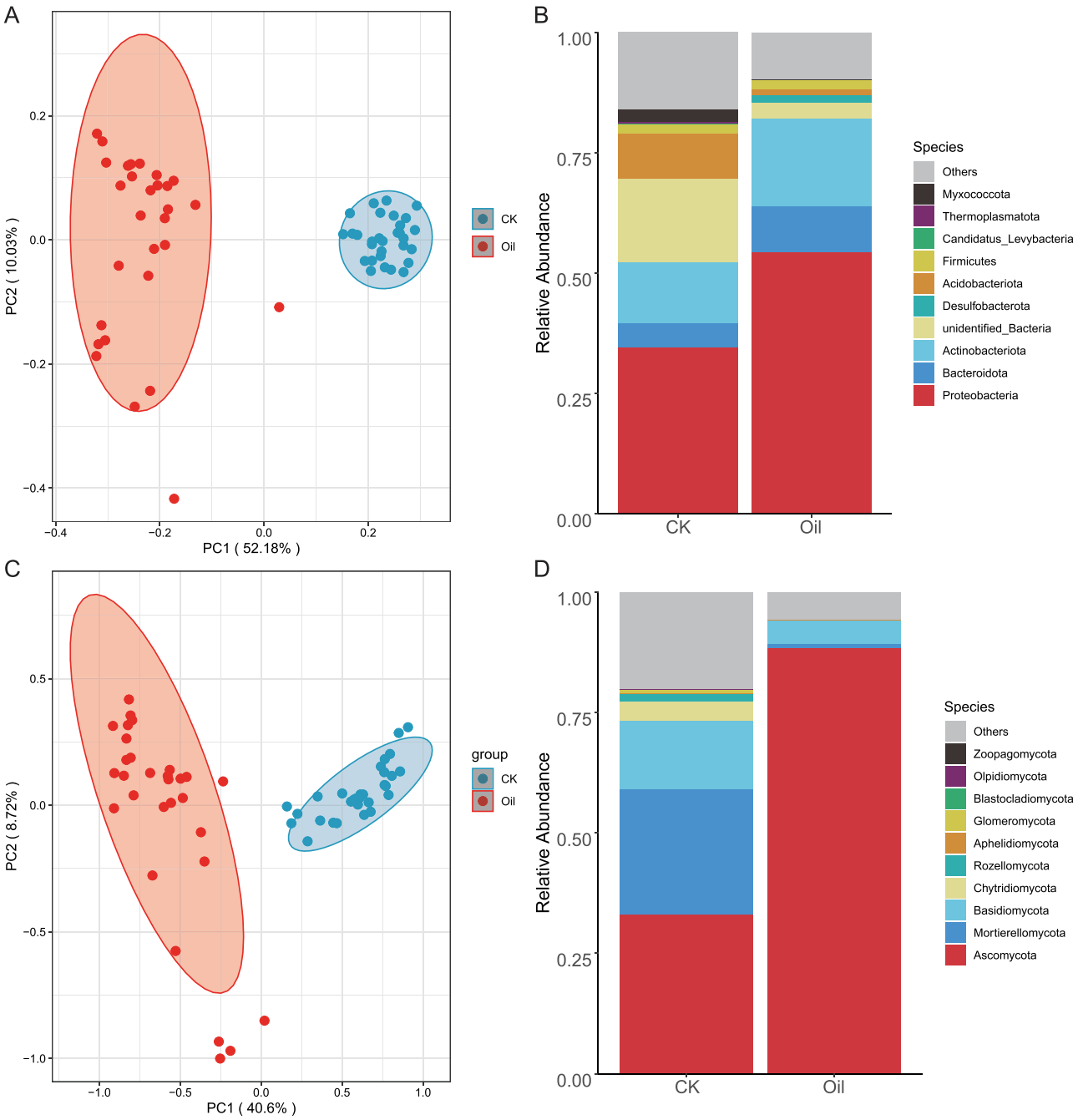

Fig. 3. The microbial composition in petroleum-contaminated soil. A PCoA plot based on weighted UniFrac distances was coloured for the Oil and CK groups of the bacterial community. The significant differences between groups were calculated by analysis of similar (ANOSIM) tests; B The relative abundance of the top 10 phyla in Oil and CK groups of bacterial microorganisms; C PCoA plots based on weighted UniFrac distances were coloured for the Oil and CK groups of fungi community. The significant differences between groups were calculated by analysis of similar (ANOSIM) tests. D The relative abundance of the top 10 phyla in the Oil and CK groups of fungal microorganisms.

Acidobacteria and 5.05% *Bacteroidota*; the bacterial microbiota of the Oil group was composed of 54.48% *Proteobacteria*, 18.1% *Actinobacteria*, 3.4% *Bacteria*, 1.3% *Acidobacteria* and 9.45% *Bacteroidota* (Fig. 3B). The above results showed that petroleum pollution changed the specific composition of the soil microbial community; significantly promoted the proliferation of *Proteobacteria, Bacteroidota* and *Actinobacteriata*; changed the dominant microbial community in the original soil; and formed a distribution dominated by *Proteobacteria*. We further analysed the composition of bacterial microflora at the genus level (Fig. S1) and found that the CK group was composed of 7.2% *Sphingomonas* and other microflora, while the Oil group was composed of 11.4% *Pseudomonas*, 11.1% *Pseudoxanthomonas*, 3.8% *Immundisolibacter*, 3.8% *Proteophilum* and 3.7% *Halomonas*. According to the difference analysis of the relative abundance of bacteria, we found that oil significantly increased the relative abundance of *Pseudomonas* and *Pseudoxanthomonas*, indicating that oil pollution could promote the proliferation of *Pseudomonas* and *Pseudoxanthomonas* in soil and make them the dominant flora.

We also analysed the PCoA of the fungal microbiota and found that there were significant differences in the overall microbial composition of fungi between the CK group and Oil group (Fig. 3C). The PC1 distribution with the largest contribution rate revealed changes of 40.6%, and the PC2 distribution revealed changes of 8.72%. The clustering difference between the two groups of samples was obvious. In conclusion, the above results showed that oil pollution can significantly change the overall composition of bacteria and fungi in soil. We also analysed fungal microorganisms at the phylum level in the oil-contaminated soil (Fig. 3D). The fungal microbiota of the CK group was composed of 33.1% *Ascomycota*, 26.1% *Mortierellomycota*, 14.1% *Basidiomycota* and 4% *Chytridiomycota*, while that of the Oil group was composed of 88.3% *Ascomycota*, 1% *Mortierellomycota* and 4.8% *Basidiomycota*. We found that the relative abundance of *Ascomycota* in the Oil group was higher than that in the CK group, while *Mortierellomycota* and *Basidiomycota* were lower than that in the CK group. The significance of differences between groups was analysed based on a t test. The above results indicated that oil-contaminated soil can promote the proliferation of *Ascomycota* at the phylum level of soil fungi and inhibit the growth of *Mortierellomycota* and *Basidiomycota.* We further analysed the fungal microbial composition at the genus level (Fig. S1) and found that the fungal microbiota of the CK group was composed of 17.1% *Mortierella* and 3% *Conocybe*, while the fungal microbiota of the Oil group was composed of 13.1% *Aspergillus*, 8.9% *Scedosporium*, 9.8% *Pseudolescheria*, 9.8% *Pseudogymnoascus* and 17.7% *Acremonium*. The relative abundances of *Aspergillus, Pseudoallescheria, Pseudogymnoascus* and *Acremonium* in the Oil group were higher than those in the CK group, and the abundance of *Mortierella* in the Oil group was lower than that in the CK group. The results showed that there were significant differences between the dominant fungal microorganisms in oil-contaminated soil and nonoil-contaminated soil at the genus level, and oil pollution

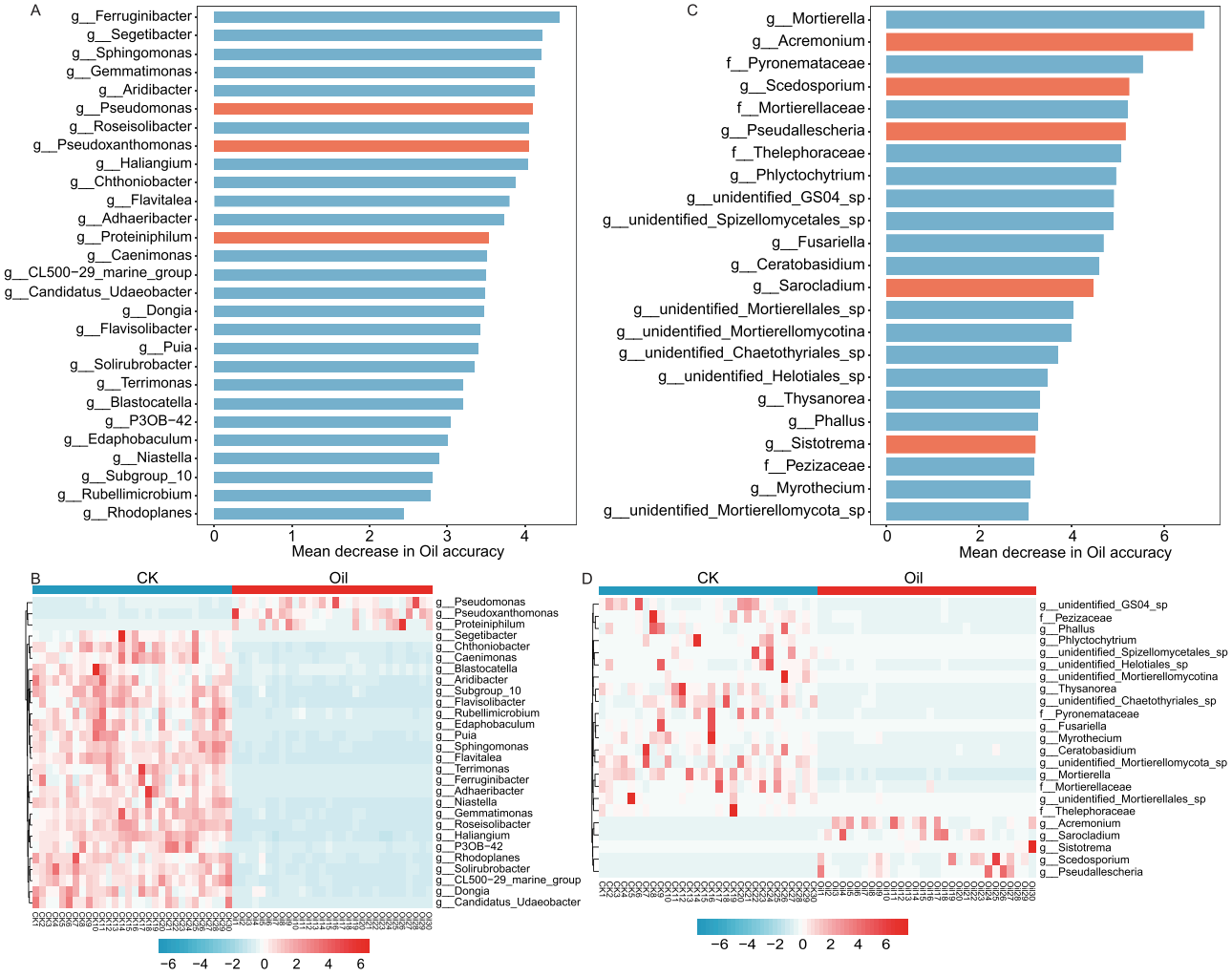

Fig. 4. Genera are strikingly different across the CK and Oil groups. A-B The 28 most discriminant genera in the models classifying the CK and Oil groups of bacterial microorganisms at the genus level. The bar lengths indicate the importance of the variable, and the colours represent enrichment in CK (green shades) or Oil (red shades). C-D The 23 most discriminant genera in the models classifying the CK and Oil groups of fungal microorganisms at the genus level. The bar lengths indicate the importance of the variable, and the colours represent enrichment in CK (green shades) or Oil (red shades).

promoted the proliferation of *Aspergillus, Pseudoallescheria, Pseudogymnoascus* and *Acremonium*.

3.4. Differential biomarkers of bacterial and fungal microorganisms in oil-contaminated soil

To explore the differences in biomarkers between the two groups, we developed a random forest model based on the differential genera with a relative abundance > 1 in at least 50% of the samples from the CK and Oil groups. The optimal model utilized 28 genera that provided the best discriminatory power (Fig. 4A). The optimal model primarily consisted of members of *Ferruginibacter, Segetibacter, Sphingomonas,* and *Gemmatimonas*. We also found from the corresponding heatmap that the relative abundance of a large number of microorganisms at the genus level in the oil group showed a significant downwards trend (Fig. 4B), such as *Segetibacter, Chthoniobacter, Caenimonas,* and *Blastocatella,* while *Pseudomonas*, *Pseudoxanthomonas* and *Proteiniphilum* increased significantly. The above results showed that oil contamination of soil will cause a sharp decline in a large number of specific bacteria, inhibit the proliferation of some bacterial microorganisms, and promote the enrichment of petroleum-degrading bacteria such as *Pseudomonas*, significantly changing the dominant flora, which is also consistent with the results in Section 3.3.

We also used the random forest model to explore the biomarkers of fungal microorganisms in the CK group and Oil group at the genus level. The optimal model utilized 23 genera that provided the best discriminatory power (Fig. 4C). The genera present in the optimal model included *Mortierella, Acremonium, Pyronemataceae,* and *Scedosporium.* We found that the Oil group also significantly reduced the relative abundance of a large number of flora in fungal microorganisms (Fig. 4D), such as *Pezizaceae, Phallus, and Phlytochytrium*; that is, oil-contaminated soil significantly inhibited the proliferation of a large number of fungal microorganisms, resulting in a significant decline in their abundance, and selectively promoted the relative abundance of dominant fungi, such as *Acremonium, Sarocladium, Scedosporium* and *Pseudoallescheria*, which promoted the transformation of dominant fungi.

3.5. Changes in the cooccurrence network relationship between bacteria and fungi in oil-contaminated soil

In the above results, we found that oil-contaminated soil significantly reduced the relative abundance of most bacterial and fungal microorganisms in the soil and shaped their unique dominant flora. To further explore the changes in colonies in the soil, we analysed the symbiotic network relationship between the CK group and the Oil group based on Spearman's test. From Fig. 5A and B, we found that the symbiotic relationship between colonies in oil-polluted soil was significantly reduced, and the symbiotic relationship between microorganisms in nonoil-polluted soil was strong. The number of nodes in the CK group was 192, and the number of edges was 778, while the number of nodes after oil pollution was gradually reduced to 150, and the number of edges was gradually reduced to 174. At the same time, we found that the oil-contaminated soil changed the symbiotic network relationship originally dominated by *Bradyrhizobium, Actinoplanes, Microvirga, Actinomycetospora* and other bacteria, forming the mutual relationship of the symbiotic flora dominated by *Geoalkalibacter, Pontibacillus, Derxia* and *Thalassosspira*.

The above results showed that after oil contaminated the soil, not only was the symbiosis between the bacterial colonies in the soil significantly reduced but also a new symbiosis relationship dominated by *Geoalkalibacter* was formed.

3.6. Correlation analysis between physical and chemical indices and soil microbiota

We correlated the three levels with the highest relative abundance of bacterial microorganisms with environmental factors (Fig. 6A, B). A Mantel test was conducted between environmental factors and the three levels with the highest relative abundance of bacterial microorganisms in the soil. We found that before oil pollution, TN was the most significant factor affecting the microbial community composition at the level

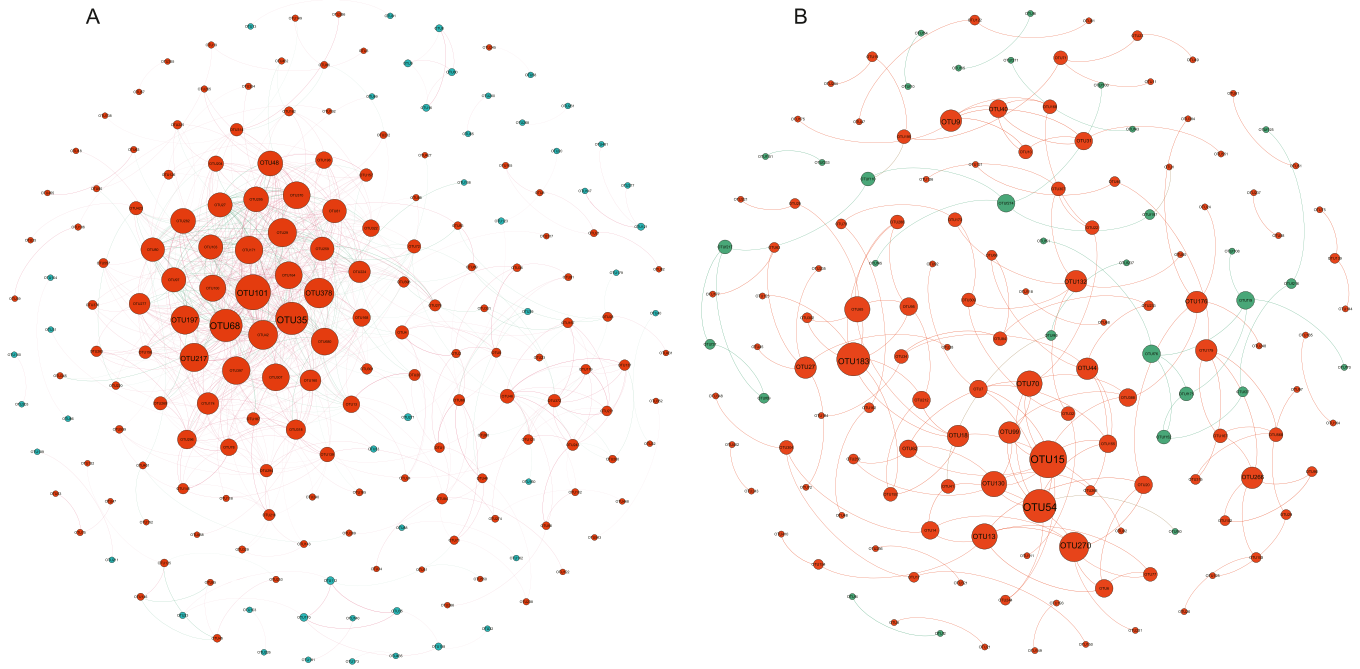

Fig. 5. Co-occurrence network analysis of soil microorganism communities in oil-contaminated and uncontaminated groups. A and B are cooccurrence networks coloured by genus and represent the CK and Oil groups, respectively. The size of the node is proportional to the relative abundance of the genus. Each connection shown has a Spearman's coefficient > 0.6 and a p value < 0.05. Red indicates bacteria, green indicates fungi, the red line is a positive correlation, and the green line is a negative correlation.

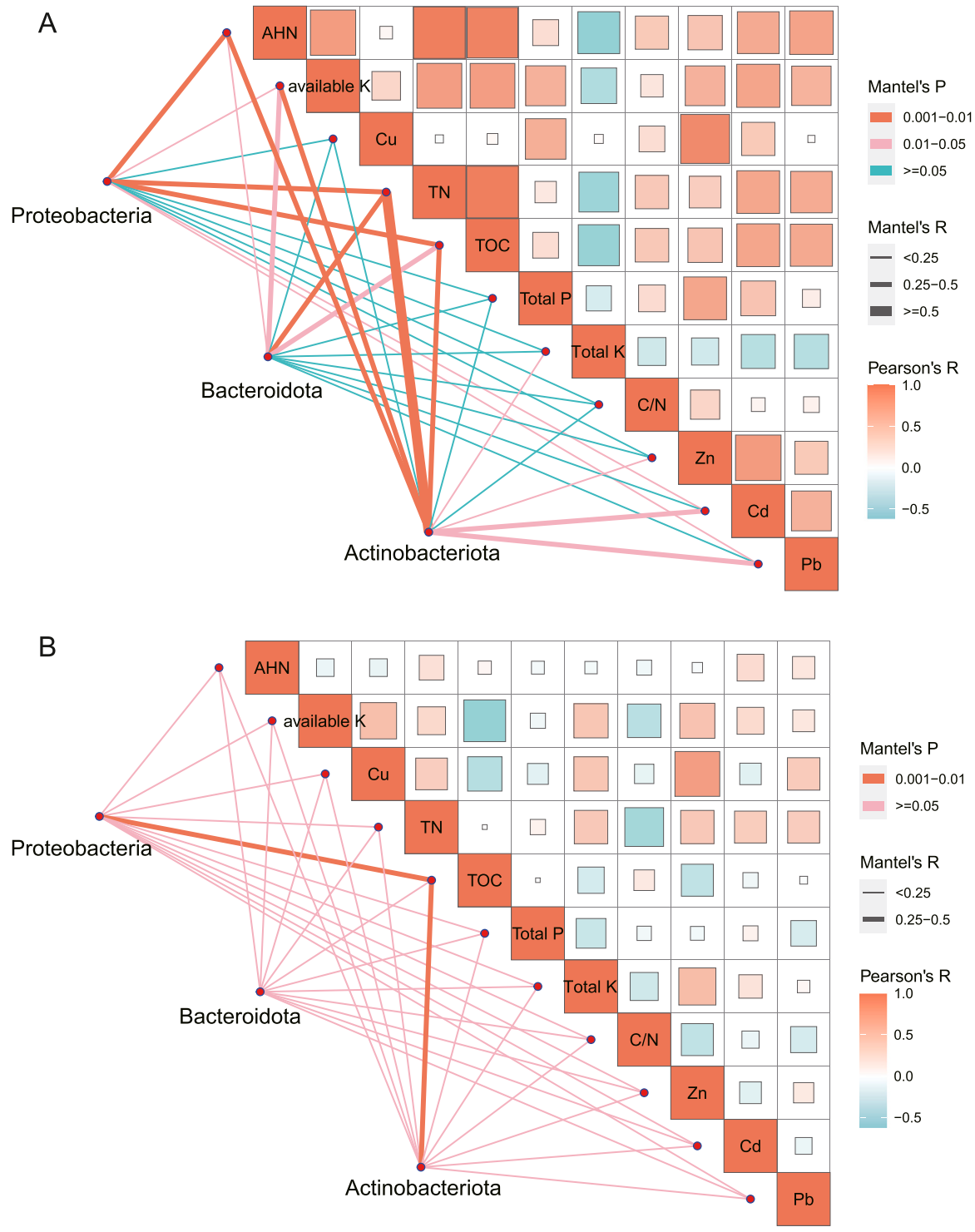

Fig. 6. Environmental drivers of soil microbial community composition. Pairwise comparisons of environmental factors with a colour gradient denoting Spearman's correlation coefficients are shown; A and B represent the CK and Oil groups, respectively. A Mantel test was conducted between environmental factors and the three levels with the highest relative abundance of bacterial microorganisms in the soil. Edge width corresponds to Mantel's r statistic for the corresponding distance correlations, and edge colour denotes the statistical significance.

of the main bacterial phyla in the soil; that is, TN had the strongest correlation with the microbial community composition of the CK group, followed by AHN, TOC and available K. After oil pollution, TOC was the most significant factor affecting the microbial community composition at the level of the main bacterial phyla in the soil. That is, TOC has the strongest correlation with the microbial community composition of the Oil group. The above results show that TOC is a strong correlation factor affecting the composition and change of soil microorganisms and a key driving factor of change to oil contaminated soil microorganisms.

4. Discussion

Microorganisms are decomposers in ecosystems that play an indispensable role in material exchange and energy transfer in ecosystems.

However, oil pollution affects the growth and reproduction of many kinds of microorganisms, resulting in changes in soil microbial species diversity (Fan et al., 2020). Regarding the effect of oil pollution on soil bacterial diversity, different research objectives have had different conclusions. Li et al. (2015) found that there was a positive correlation between soil oil content and bacterial diversity in the Daqing Oilfield and Karamay Oilfield. Bidga et al. (Bidja Abena et al., 2020) found that unpolluted soils were more diverse and evenly distributed than contaminated samples. This conclusion is consistent with the results of this study. We found that oil pollution significantly reduced the abundance and diversity of bacterial fungi in the soil and significantly changed the dominant bacteria in the soil microbial composition, forming bacterial microbiota with *Proteobacteria, Actinobacteria* and *Bacteroidota* as the main dominant bacterial phylum and *Ascomycota* as the main dominant fungal phylum.

At present, *Proteobacteria, Actinobacteria, Bacteroidota and Firmicutes* have been used in the biodegradation of different hydrocarbons in petroleum-contaminated soil, including aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and asphaltenes (Liu et al., 2020). Fungal bioremediation is considered a promising technology to remove PAHs from contaminated sites (Li et al., 2021b). *Sphingomonas, Sphingobacterium, Acidovorax, Massilia, Flavobacterium, Cupriavidus, Aeromicrobium* and unclassified *Chitinophagaceae* were thought to be related to the clearance of PAHs. Fungi participate in the cometabolism of functional protein bacteria and promote the degradation of PAHs, which indicates that the biodegradation of PAHs is the result of the cometabolism of fungi and bacteria. The conclusions provide a more comprehensive view to support the process of fungal bioremediation of soil. The above results show that it is very important to study and understand the microbial composition of oil-contaminated soil, which can provide an important reference for the biological methods of soil remediation. In addition, it has been proven that *Pseudomonas* is the dominant group in soil polluted by hydrocarbons (oil or gasoline, etc.) (Li et al., 2020a). Lopes Ferreira et al. (2006) found that the crude oil-degrading bacteria were mainly *Pseudomonas, Blastococcus* and *Nocardioides*, which is consistent with the results of this study.

This study also found that the total organic carbon and carbon-nitrogen ratio of the soil increased significantly after oil pollution. Previous studies have shown that when the total petroleum hydrocarbon content in soil increases, that is, the oil in soil pores is dominant and its saturation is higher, the water content in soil pores is lower, and vice versa. The strong hydrophobicity of oil leads to the hydrophobicity of soil with high oil content, resulting in the decrease of soil water content, the decrease of soil water storage capacity, and the accumulation of soil salt (Haghollahi et al., 2016). The number of *Actinomycetes* in the soil increased, which led to an increase in acid substances produced by the anaerobic degradation of microorganisms, resulting in a decrease in the soil water content (Koshlaf et al., 2020; Li et al., 2021a). We also found that the relative abundance of *Actinomycetes* in contaminated soil increased significantly, which was consistent with this conclusion. When petroleum enters the soil environment, it reduces soil aeration and provides an abundant carbon source for soil microorganisms, which strengthens the natural degradation process of petroleum hydrocarbons by anaerobic bacteria in soil. In the process of anaerobic degradation, sulfate-reducing bacteria and other anaerobic bacteria are prone to produce acidic substances (Dell'Anno et al., 2009), which can change the pH value of soil and affect the composition and metabolism of the microbial community. Most of the microorganisms that can metabolize petroleum hydrocarbons have the strongest activity under the conditions of neutral pH 7 and a soil C/N ratio of 100/10 (Farkas et al., 2015). It was found that the C/N ratio of soil contaminated by petroleum was significantly higher than that of nonpetroleum-contaminated soil. Therefore, adding a nitrogen source to oil-contaminated soil is a feasible bioremediation method in theory. Wu et al. (2019) explored the benefits of biostimulation with nitrogen and phosphorous (BS) in terms of petroleum hydrocarbon removal and microbial community structure change in petroleum-polluted soil and found that after 12 weeks of remediation, the removal rate of TPH was significantly increased, and the composition of the microbial community was more abundant; this indicated that an abundance of nitrogen and phosphorus nutrients could ensure the activity of degradation agents in oil-contaminated soil, and the structure and diversity of the microbial community in soil were the key factors affecting the biodegradability of oil.

We also used correlation analysis of environmental factors to study the interdependent relationship between microbial community structure and environmental factors in oil-contaminated soil and further explained the role of environmental factors on the distribution of the soil microbial community in different environments. Wu et al. (2022) through CCA analysis showed that TN, TP and TOC had significant effects on bacterial community structure, especially TOC content, which was closely related to the distribution of *Pseudomonas*. The results showed that the diversity index and spatial distribution of the oil-contaminated soil community were affected by soil environmental parameters. This is also consistent with our results. We found that the TOC content in oil-contaminated soil plays a regulatory role in the remodelling and composition of bacterial flora in oil-contaminated soil. Chayabutra and Ju (2000) found that the concentration of dissolved TOC increased in the continuous biodegradation of cetane by *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was able to convert hydrocarbons used for cell growth into oxygenated organic compounds through cell metabolism, resulting in an increase in TOC levels. Margesin et al. (2007) found that the total number of bacteria increased several times in a short time after adding N, P and other nutrients to the oil-contaminated area, and the contents of n-alkanes and isoprene decreased rapidly. All these results indicate the importance of environmental factors to the remediation of petroleum-contaminated soil by petroleum-degrading bacteria. In this paper, the environmental factors and the structure of the soil microbial community were effectively explored in depth, revealing that TOC plays an important role in regulating the composition of bacterial microbiota in oil-contaminated soil.

5. Conclusion

In conclusion, oil-contaminated soil has good bioremediation, so we should fully develop the resources of indigenous bacteria and add nitrogen and other nutrients to stimulate the growth of indigenous oil-degrading bacteria and improve the oil degradation rate. After oil pollution, the abundance of soil microorganisms, especially oil-degrading microorganisms, increased rapidly and formed a unique dominant position, providing a large number of flora resources for the bioremediation of oil-contaminated soil. Additionally, a certain concentration of oil can promote the growth of some microorganisms, add exogenous nutrients, and make the dominant bacteria in the process of bioremediation of oil-contaminated soil, which can play the role of degrading petroleum pollutants.

CRediT authorship contribution statement

Y.L., C. L. and Y. X. designed the research; Y.L., T. H. and J.L. performed the sample collection; Y.L. and C. L. assisted with the 16 S rRNA gene sequencing data analysis; Y.L. and C. L. wrote the paper and performed the experiments. The authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The present study data are available from the corresponding author upon reasonable request.

Acknowledgements

This research was supported by National Natural Science Foundation of China (NSFC) (Grant No. 31760026 and 81260411). Novogene Bioengineering company is gratefully acknowledged for DNA sequencing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114129.

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